

Solvent enhanced ion chromatography of alkaline earth and transition metal ions on porous monolithic silica

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Abstract

The high-performance separation of alkaline earth and transition metal ions on a bare porous silica 10.0 cm × 0.46 cm monolith (Merck Chromolith Si) was achieved using solvent enhanced ion chromatography. Using acetonitrile or methanol based ammonium and sodium acetate buffered mobile phases, the effect upon cation retention of mobile phase ionic strength, organic content and pH were evaluated. Increasing mobile phase acetonitrile concentration from 60 to 80% acetonitrile resulted in 10–15-fold increases in cation retention due to reduced hydration of the metal ions and hence enhanced ion-exchange interactions with surface silanol groups. The optimised conditions for the separation of Cu(II), Cd(II), Ni(II), Co(II) and Mn(II) were found to be 80% MeCN with 10.3 mM ammonium acetate (pH 4.6). Detection of transition metal ions was obtained using post-column reaction with 4-(2-pyridylazo) resorcinol and absorbance at 510 nm. Under elevated flow conditions (up to 5 mL/min), total runs times could be reduced to under 4 min. Optimum peak efficiencies were seen at flow rates of between 1.5 and 2 mL/min and were equivalent to an average efficiency of 25,000 N/m.

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1. Introduction

Since the first paper describing the application of porous silica rods for HPLC was published by Tanaka and co-workers [1], scientists have been motivated to study their performance and possible application [2]. The unique properties of monoliths, in particular their tolerance to high flow rates whilst maintaining excellent peak efficiencies, and the rapid speed of chromatographic separations that can be achieved at acceptable backpressures, make the monolithic column format more suitable to some applications than commonly used micro-particulate packed columns. However, due to the fact that monolith column technology is still relatively new, the number of different stationary phase chemistries and separation modes remains much smaller than that available for packed columns. In particular, both polymer and

silica based monolithic columns have overwhelmingly been applied to reversed-phase separations only, and have received less attention in other modes of LC, including ion exchange and ion chromatography (IC). However, a limited number of research groups have recently been exploring the potential use of monolithic phases for rapid high-performance separations of smaller ionic species with some considerable success, including application to separations of inorganic anions and inorganic cations [3–12]. The majority of these early studies have used liquid ion-exchangers dynamically coated onto reversed-phase monoliths [3–5,7,10], although a few papers have now appeared describing covalently bonded ion-exchangers based upon monolithic supports [6,11,12] and even agglomerated porous monolithic substrates [8,9]. An early review of all of the above work, describing new possibilities in ion analysis using monolithic columns has recently been compiled [13].

In a recent study, Pack and Risley described the use of a bare silica porous monolith to separate a number of alkali

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metal cations using an ammonium acetate buffer mobile phase containing up to 90% acetonitrile. Detection was achieved using evaporative light scattering detection (ELSD) and the technique was applied to the determination of alkali metal counter ions in a number of pharmaceutical preparations [14]. The authors described the separation as being based upon hydrophilic interaction chromatography (HILIC). The term hydrophilic interaction chromatography has been assigned to LC that is based upon the use of polar stationary phases with less polar mobile phases. Usually, the HILIC mobile phase contains a high percentage of an organic solvent, typically 60–90% acetonitrile and a 10–40% water or aqueous buffer. Under these conditions the role of weak interactions like dipole–dipole, ion–dipole and hydrogen bonding between the analyte and stationary phase is remarkably enhanced and results in stronger retention of polar solutes. Theory of HILIC also states that retention can be governed through the partition of polar analytes into an enriched stagnant water layer on the stationary phase surface. Through increasing the organic content of the mobile phase, this partition coefficient is increased for polar solutes, resulting in increased retention [14]. The retention behaviour seen with HILIC is the opposite to that of reversed-phase liquid chromatography and similar to normal phase selectivity, although the high water content of the mobile phase means improved solubility for polar analytes. To-date HILIC has seen most application to small charged biomolecules.

In the case of the separation of small ions on polar stationary phases, as in the experiments described by Pack and Risley using porous monolithic silica [14], the above explanation of retention behaviour may not be entirely suitable. Pack and Risley showed that retention of the alkali cations was dependent upon % organic solvent, ionic strength and mobile phase pH. The effect of increasing ionic strength of the mobile phase was to decrease retention, clearly due to weak ion-exchange interactions with the surface silanol groups. In addition, an increase in mobile phase pH, over the range 3.5–6.5, saw a subsequent increase in retention, presumably again due to the dissociation of surface silanol groups. This was the basis of the assumption that the retention mechanism was based upon HILIC.

However, retention of alkali and alkaline earth metal ions on bare silica supports has been shown in many previous studies [15–21]. In these cases, weak ion-exchange interactions were responsible for ion retention. Under conditions of high concentrations of organic solvent within the mobile phase such interactions will become stronger due to a reduced number of waters of hydration associated with the ion. This effect is commonly seen within inorganic ion exchange [18,19,22]. In cation exchange, increasing mobile phase organic solvent content has been shown to cause significant changes. The exact effect depends on the dielectric constant of organic solvent and its ability to form hydrogen bonds. For example, the increase of alcohol content in the mobile phase has actually been shown to cause a decrease in retention times of alkali and alkaline metal cations on silica, due to interac-

tions with silanols groups and a corresponding decrease in its ion-exchange capacity [20]. Alternatively, an increase of acetonitrile content in water based mobile phases has been shown to produce the opposite effect of increasing retention [18,19]. With this in mind, it is therefore proposed here that what was actually observed by Pack and Risley [14] was in fact solvent enhanced ion-exchange interactions and, correspondingly the separation mode is solvent enhanced ion chromatography (SEIC) rather than HILIC.

In the work presented here, the solvent affect described by Pack and Risley [14] is investigated further through application to alkaline earth and transition/heavy metal cations. The ability to efficiently and rapidly separate the full suite of common transition and heavy metal cations is still a challenging task and the ability to utilise simple silica monoliths for this purpose has clear advantages [23].

2. Experimental

2.1. Chromatographic conditions

A Dionex Model GPM2 Gradient Pump Module (Sunnyvale, CA, U.S.A.) was used to deliver the eluent (1.0–4.0 mL/min). An automated injection valve, fitted with a 20 μ L injection loop was used for the introduction of standards. A PEEK lined bare silica monolithic column (Performance SI) of 10 cm in length and 4.6 mm i.d. was purchased from Merck KGaA (Darmstadt, Germany). According to the manufacturer the silica monolith had a surface area of 300 m² g⁻¹, with a 2 μ m macroporous and 13 nm mesoporous structure. A pressure driven Dionex Reagent Delivery Module was used for the introduction of the PCR, which was mixed at room temperature with the eluent using a 0.5 m PEEK reaction coil, 0.25 mm i.d. (the 0.5 m reaction coil was required to ensure adequate mixing of the eluent and PCR at elevated flow rates). An applied Biosystems 400 Solvent Delivery System (Foster City, CA, U.S.A.) was used to deliver the PCR when eluent flow rates greater than 3 mL/min were employed. A 1050 series, Hewlett-Packard UV–vis detector (Palo Alto, CA, U.S.A.) was used to monitor the resultant chromatograms. When working with eluent flow rates >3.5 mL/min, a Waters Model 600E Multisolvent Delivery System (Waters, Milford, MA, U.S.A.) was used as the eluent delivery system, and a Shimadzu Model SPD-6AV UV–vis detector (Kyoto, Japan) was used to monitor the resultant chromatograms. Data acquisition was at a rate of 10 Hz with processing of chromatograms performed using a PeakNet 6.0 chromatography workstation (Dionex).

2.2. Reagents

Acetonitrile, methanol (HPLC grade, LabScan Ltd., Stillorgan Ind. Park, Dublin), ammonium acetate, sodium acetate (Aldrich Chemicals Ltd., Gillingham, UK) and glacial acetic acid (Merck KGaA) were used to prepare the required

eluents. All eluents and standard solutions were prepared using deionised water from a Millipore Milli-Q water purification system (Bedford, MA, U.S.A.), and were filtered through a 0.45 μm filter and degassed by sonication. The PCR reagent used for the detection of transition metals consisted of a mixture of 0.4 mM 4-(2-pyridylazo) resorcinol (PAR, purchased from Sigma–Aldrich, Gillingham, UK) and 0.5 M ammonia (BDH Laboratory Supplies, Poole, England), adjusted to pH 10.5 (monitored at 510 nm). For the detection of alkaline earth metals the PCR reagent used was 0.4 mM *o*-cresolphthalein complexone (*o*-CPC, Sigma–Aldrich), 0.25 M boric acid (Sigma–Aldrich, Tallaght, Dublin, Ireland) adjusted to pH 10.5 with 1 M NaOH (monitored at 570 nm). Low-level standard solutions of metal cations were generally made up freshly each day from stock solutions (1000 ppm). Mg(II), Ca(II), Sr(II), Ba(II), Mn(II), Co(II), Ni(II), Cd(II), Zn(II) and Pb(II) standards were prepared from their chloride salts (Sigma–Aldrich) and Fe(II) and Cu(II) standards were prepared from their sulphate salts (Sigma–Aldrich).

3. Results and discussion

3.1. Alkaline earth metals—organic solvent and buffer effects

Initial experiments were performed to extend the work of Pack and Risley to include alkaline earth metal ions [14]. Therefore, using an acetic acid/ammonium acetate buffer within an acetonitrile based mobile phase, the effect upon selectivity of varying the buffer concentration within mobile phases of varying % acetonitrile was investigated. The acetonitrile was varied from 60 to 80% ($n=3$), whilst the ammonium acetate buffer concentration was varied from 10.5 to 42.1 mM ($n=4$), 7.8 to 31.3 mM ($n=4$), 5.2 to 20.6 mM ($n=4$), for 60, 70 and 80% acetonitrile, respectively. For each mobile phase prepared, the pH was constant at 4.6. Table 1 shows the retention data obtained, together with the slope and correlation coefficient data for the dependences of $\log k$ from the log of the buffer concentration, $[E]$. As can be seen from Table 1, a linear relationship between $\log[E]$ and retention was evident with each % of MeCN, and in general the slopes for all four alkaline earth metals did not vary significantly, and so no noteworthy selectivity changes were seen.

The data shown in Table 1 show considerable increases in retention for all four cations with increasing mobile phase % MeCN. Ten to 15-fold increases in retention factors (k) can be seen with an increase in % MeCN from 60 to 80%. A number of studies have investigated the effect upon ion exchange of increasing the organic character of the mobile phase on inorganic ion-exchangers [14,18–20,24]. These studies conclude that the degree of hydration of the analyte and eluent ions has a major influence upon selectivity, and that by including organic solvents within the mobile phase, both ions in solution and fixed ion-exchange sites within the stationary

Table 1

Retention data for alkaline earth metal ions on a 10 cm bare silica monolithic column using an acetonitrile mobile phase containing ammonium acetate buffer—(pH 4.6)

$\log[E]$	$\log k$			
	Ca(II)	Mg(II)	Sr(II)	Ba(II)
60% MeCN				
–1.98	0.324	0.283	0.324	0.436
–1.68	0.057	0.053	0.068	0.182
–1.50	0.000	–0.013	0.049	0.161
–1.38	–0.260	–0.284	–0.210	–0.208
Slope	–0.853	–0.884	–0.790	–0.930
R^2	0.913	0.932	0.903	0.842
70% MeCN				
–2.11	0.728	0.683	0.821	0.914
–1.81	0.430	0.418	0.509	0.635
–1.63	0.260	0.223	0.328	0.449
–1.51	0.170	0.170	0.289	0.418
Slope	–0.937	–0.884	–0.920	–0.862
R^2	0.997	0.989	0.981	0.979
80% MeCN				
–2.28	1.417	1.450		
–1.99	0.969	0.985		
–1.81	0.849	0.879		
–1.69	0.694	0.704		
Slope	–1.186	–1.211		
R^2	0.979	0.975		

Flow rate = 1 mL/min, column temperature = 20 °C and post-column reaction detection with *o*-CPC monitored at 570 nm.

phase will lose waters of hydration. In addition, selectivity will also be much affected by the extent of hydrogen bonding of the solvent molecules with surface silanol groups. In most cases, reduced hydration results in enhanced analyte ion interaction with the stationary phase exchange sites and thus increased retention. The exact nature of this effect would depend upon the relative solvation of the analyte and eluent ions and the nature of the organic solvent, namely its dielectric constant. Solvents that exhibit strong hydrogen bonding properties should result in a relatively smaller retention increase or even decrease compared to solvents that do not exhibit such properties.

At 60 and 70% MeCN, the retention order shown with the MeCN mobile phase was typical of that seen with ion-exchange, namely $\text{Mg(II)} < \text{Ca(II)} < \text{Sr(II)} < \text{Ba(II)}$. However, at 80% MeCN Mg(II) was retained for slightly longer than Ca(II). Apart from the above change in retention order, the mobile phase MeCN concentration also had relatively little effect upon individual cation selectivity, with relative retention of all metals increasing at a roughly equal rate.

Fig. 1(a) shows the overlay of three chromatograms of Mg(II), Sr(II) and Ba(II) standards obtained with a 70% MeCN mobile phase at an acetate buffer concentration of 31.3 mM (pH 4.6), delivered at a flow rate of 1 mL/min. Clearly, although peak shapes for Mg(II) and Ba(II) are good, Ca(II) and Sr(II) exhibited peak splitting, and under the conditions shown Ca(II) co-eluted with Mg(II). The close selectivity exhibited for Ca(II) and Mg(II) under different

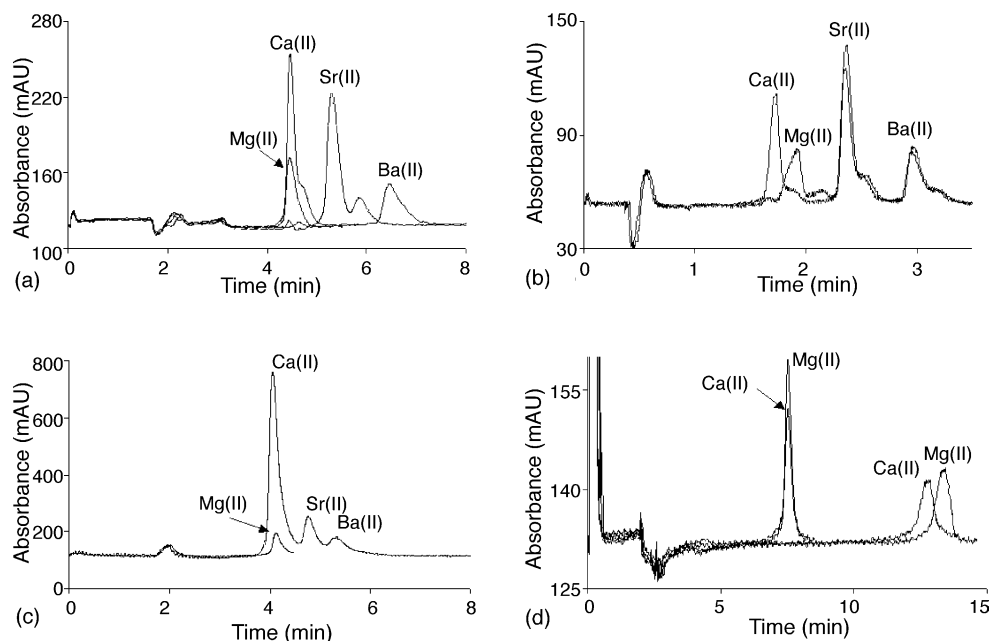


Fig. 1. Separations of alkaline earth metals on a bare silica monolith. Overlaid chromatograms of mixed alkaline earth standards obtained using a mobile phase of: (a) 70% MeCN containing 31.3 mM ammonium acetate (pH 4.6), flow rate = 1 mL/min; (b) 70% MeCN containing 23.4 mM ammonium acetate (pH 5.55), flow rate = 4 mL/min; (c) 70% MeCN containing 31.3 mM sodium acetate (pH 4.6), flow rate = 1 mL/min; (d) 80% MeCN containing 41.2 mM ammonium acetate at pH 4.9 (retention of Ca(II) and Mg(II) = 7.5 min) and pH 5.9 (retention of Ca(II) = 12.7 min, Mg(II) = 13.5 min), flow rate = 1 mL/min.

mobile phase conditions was unexpected, particularly when compared to previous studies on silica gel columns [20,21], although in most previous studies complexing eluents have been used which obviously affect the observed selectivity.

3.2. pH effects

Using a 70% MeCN mobile phase with an ammonium acetate buffer concentration of 23.4 mM, the effect upon retention of varying mobile phase pH from 4.6 to 5.55 ($n = 3$) was investigated. The results are included in Table 2. As expected, over this pH range retention times for all four cations increased and it was observed that at the higher pH values the partial separation of Ca(II) and Mg(II) was

now possible, although the peak shape for Mg(II) was poor (Fig. 1(b) shows overlays of chromatograms from mixed standards obtained with a 70% MeCN mobile phase containing 23.4 mM buffer at pH 5.55, run under an elevated flow rate of 4 mL/min). A small degree of peak splitting was now noted for all four peaks. In an attempt to eliminate this unusual peak splitting, increased buffer concentrations were investigated. Using an 80% MeCN mobile phase, the overall buffer concentration was increased to 41.2 mM (pH 4.9). Under these conditions the peak splitting evident at lower buffer concentrations was no longer apparent, although under these conditions Ca(II) and Mg(II) co-eluted once again.

Keeping these mobile phase % MeCN and buffer concentrations constant but increasing pH up to 5.94, improved the separation whilst maintaining acceptable peak shapes and retention times (data included in Table 2). Fig. 1(d) shows overlaid chromatograms for standard solutions of Mg(II) and Ca(II) with the high buffer concentration adjusted to pH 4.9 (peaks co-elute at 7.5 min) and then pH 5.94 (peaks eluting at 12.7 min—Ca(II) and 13.5 min—Mg(II)). As can be seen from Fig. 1(d) peak shapes are no longer split at higher buffer concentrations, even at increased retention times, although obviously the column selectivity for Ca(II) and Mg(II) is still very similar.

3.3. Alternative buffer

The ammonium acetate in the acetonitrile mobile phase was replaced by sodium acetate buffer (pH 4.6) and the effect of using an alternative buffer on alkaline earth selectivity was

Table 2

pH effect upon retention for alkaline earth metal ions on bare silica monolithic column using an acetonitrile mobile phase containing ammonium acetate buffer

pH	Retention factor (<i>k</i>)			
	Mg(II)	Ca(II)	Sr(II)	Ba(II)
70% MeCN, 23.4 mM buffer				
4.60	1.79	1.97	2.26	3.01
5.01	2.27	2.22	2.87	4.22
5.55	3.37	2.60	3.37	4.72
80% MeCN, 41.2 mM buffer				
4.40	3.40	3.45		
4.90	3.38	3.38		
5.44	5.96	4.66		
5.94	6.82	6.47		

Flow rate = 1 mL/min and column temperature = 20 °C.

investigated. Peak splitting was once again observed at low buffer concentrations, however, increasing the sodium acetate buffer concentration to 62.5 mM (70% MeCN) eliminated the problem of peak splitting whilst still allowing a separation of Ca(II), Sr(II) and Ba(II). There was no difference in the alkaline earth metal selectivity observed with the sodium acetate buffer, i.e., Mg(II) and Ca(II) were still observed to co-elute over the range of buffer concentrations studied (data included with transition metal data in Table 4) and the retention factors for the alkaline earth metal ions were similar to those obtained with the ammonium acetate buffer.

3.4. Transition metals—organic solvent and buffer effects

A number of studies have been carried out on the ion-exchange capacities of various forms of silica gel for divalent cations, including transition and heavy metal ions [25–32]. In many cases, these investigations have studied relatively large particle size silica gels, and have not investigated high-performance phases for ion chromatographic separations. However, a number of studies have emerged using unmodified silica gels for ion chromatographic separations [14,15,17–20], in some cases including selected transition metal ions [16,25]. In these studies, the retention, selectivity and efficiency exhibited, have been shown to be heavily influenced by the nature of the silica gel, including properties like metal content of the silica itself, and relative acidity of the silica surface.

Here, selectivity of the silica monolith for Cu(II), Fe(II), Cd(II), Ni(II), Co(II), Mn(II), Zn(II) and Pb(II) was investigated in MeCN based mobile phases, ranging again from 60 to 80% MeCN, buffered once more with varying concentrations of ammonium acetate–acetic acid (pH 4.6). To reduce potential column contamination from Fe(III), 1 mM ascorbic acid was also added to all mobile phases used to reduce Fe(III) to Fe(II). However, under the mobile phase and detection conditions used, the response for Fe(II) was very poor, eluting as only a small peak between Cu(II) and Cd(II). As with the alkaline earth metal ions, significant retention of each of the above metals was noted, with the retention order being Cu(II) < Fe(II) < Cd(II) < Ni(II) < Co(II) < Mn(II) < Pb(II) < Zn(II).

Interestingly, this retention order is quite different from those reported by others on bare silica gel columns. According to Iler [32], the adsorption of transition metal ions on silica is due to interaction with active sites at the silica surface which act like ligands, and the specificity of adsorption is therefore related to the tendency of the metal atom to form covalent bonds. Schindler et al. [31] supports this theory having found that the ligand properties of surface silanol groups for transition metal ions (Fe(III), Cu(II), Cd(II) and Pb(II)) strongly correlate with known stability constants for corresponding metal–hydroxocomplexes. In fact, this stability data are in good agreement with selectivity reported in several papers [28,30,31], where the fol-

lowing order of specificity of formation of covalent bonds with silica surface was observed for transition metals: Cd(II) < Ni(II) < Zn(II) < Co(II) < Cu(II) < Pb(II).

The use of bare silica particle columns for separation of Cu(II), Cd(II), Pb(II) and Tl(I) ions was also reported by Janos et al. [25], who used sodium acetate, sodium tartrate and sodium hydroxybutyrate aqueous solutions of different pH and concentration. The authors found that selectivity of separation depends strongly on secondary complexation equilibria with organic acids from the eluents. Here, with the monolithic silica column, the mobile phase contained ammonium acetate and so both ammonium and acetate could affect observed selectivity through complexation. The most obvious deviations from the above data were firstly the retention of Cu(II), which was relatively low under all mobile phase conditions and was least affected by increasing the concentration of MeCN. Secondly, was the fact that Zn(II) was strongly retained under all conditions, only eluting under conditions of low MeCN (60%) and high buffer concentration. Even under such conditions, Zn(II) eluted as an excessively broad and tailed peak. This unusual selectivity and poor peak shapes for Zn(II), on similar silica monolithic columns (Merck Performance-Si) has been specifically noted in two previous studies, one where the silica monolith was modified with iminodiacetic acid [11,12], the second where the silica monolith was modified with 2,6-diaminohexanoic acid [33]. Obviously, the results here would indicate that the effects reported in the above two studies originate from the monolith backbone itself, rather than the attached functionalities. The unusual behaviour of Cu(II) could be due to the formation of Cu(II)–MeCN complexes, leading to reduced retention. This is supported by data [34], which prove a specific solvation of Cu(II) ions by MeCN molecules.

Table 3 shows the retention data as a function of % MeCN and buffer concentration, obtained from investigating 15 different mobile phase conditions. Clearly, as with alkaline earth metal ions, retention of transition metal ions can be drastically increased using high concentrations of MeCN within the mobile phase. In addition, the selectivity shown for each cation was sufficient to result in a number of possible separations. Overlaid chromatograms of individual standards are shown in Fig. 2, with Fig. 2(a) showing retention with a 70% MeCN mobile phase containing 15.6 mM ammonium acetate buffer (pH 4.6). Fig. 2(b) shows the retention of the same standards (Pb(II) and Zn(II) retained completely) with an 80% MeCN mobile phase, containing a 5.2 mM ammonium acetate buffer (pH 4.6), and although the chromatograms show some evidence of either peak fronting or tailing, baseline resolution of the cations was easily achieved.

3.5. Alternative solvents

Clearly, the above results suggested the nature of the organic solvent may have been an important factor in the observed selectivity. To investigate this further a brief study was undertaken using MeOH in place of MeCN. MeOH

Table 3

Retention data for transition metal ions on a 10 cm bare silica monolithic column with MeCN or MeOH based mobile phases containing varying amounts of ammonium acetate buffer—(pH 4.6)

log[E]	log k					
	Cu(II)	Cd(II)	Ni(II)	Co(II)	Mn(II)	Pb(II)
60% MeCN						
–2.38	–0.12	0.53	0.56	0.58	0.62	0.84
–1.98	–0.35	0.04	0.19	0.22	0.24	0.59
–1.68	–0.52	–0.30	–0.13	–0.10	–0.07	0.22
–1.50	–0.60	–0.53	–0.30	–0.26	–0.26	0.00
Slope	–0.551	–1.188	–0.988	–0.964	–1.00	–0.967
R ²	0.999	0.999	0.999	0.999	0.999	0.971
60% MeOH						
–1.68	–1.50	–0.67			–0.56	–0.28
70% MeCN						
–2.51	0.06	0.78	0.83	0.86	0.93	
–2.11	–0.26	0.28	0.48	0.55	0.59	1.09
–1.81	–0.40	–0.02	0.24	0.33	0.37	0.78
–1.63	–0.52	–0.26	0.02	0.09	0.11	0.50
Slope	–0.650	–1.162	–0.904	–0.852	–0.906	–1.213
R ²	0.987	0.998	0.995	0.986	0.985	0.986
70% MeOH						
–1.81	–0.21	–0.53			–0.40	0.06
80% MeCN						
–2.68	0.16	1.23		1.36	1.51	
–2.28	0.02	0.85	0.98	1.11	1.24	
–1.99	–1.16	0.46	0.67	0.81	0.88	1.33
–1.80	–0.22	0.31	0.57	0.71	0.74	1.19
–1.69	–0.25	0.20	0.47	0.59	0.63	1.10
Slope	–0.443	–1.057	–0.849	–0.786	–0.912	–1.087
R ²	0.990	0.995	0.982	0.992	0.990	0.967
80% MeOH						
–1.99	0.19	–0.37	–0.32	–0.28	–0.15	0.54
90% MeOH						
–2.29	0.89	–0.03	–0.005	0.11	0.32	

Flow rate = 1 mL/min, column temperature = 20 °C and post-column reaction detection with PAR monitored at 510 nm.

is known to form strong hydrogen bonds with the surface silanol groups and so it was expected that relative retention should be less in this case. In addition, the solvation of metal ions with MeOH molecules is different as compared with MeCN, and so this should also alter the observed selectivity. Mobile phases containing 60–90% MeOH were investigated, each containing varying amounts of ammonium–acetate buffer. The resultant retention data have been included in Table 3 for comparison with MeCN data. As can be seen from the tabulated data, under similar buffer concentration, retention times were generally significantly lower with the MeOH based mobile phases compared with MeCN. Exceptions to this observation were the retention of Zn(II), which was once more selectively retained, and the behaviour of Cu(II), which showed a rapid increase in retention under high MeOH concentrations, such that at 80 and 90% MeOH the retention order was now found to be Cd(II) < Ni(II) < Co(II) < Mn(II) << Cu(II). These

observations support the above assumption that selective Cu(II)–MeCN interactions are primarily responsible for the relatively low retention of Cu(II) with MeCN based mobile phases.

Peak shapes for transition/heavy metal ions with the MeOH mobile phases were generally acceptable, with no signs of the peak splitting seen with the alkaline earth metal ions. Overall selectivity was also sufficient to achieve several useful separations of important metals. Fig. 3 shows some overlaid standard chromatograms, achieved using an (a) 60% MeOH, 21.1 mM ammonium acetate buffer mobile phase (pH 4.7), (b) 80% MeOH, 15.6 mM ammonium acetate buffer mobile phase (pH 4.7) and (c) a 90% MeOH, 5.1 mM ammonium acetate buffer mobile phase (pH 4.7).

3.6. Alternative buffers

Using the 80% acetonitrile mobile phase, the use of a sodium acetate buffer concentration over the range 10.3–41.2 mM (pH 4.6) was investigated and the effect on transition metal ion retention is shown in Table 4. The retention of all the transition ions studied increased significantly when the sodium acetate buffer was employed. This difference in retention can be attributed to complex formation, which may occur with the ammonium in the MeCN/ammonium acetate buffer mobile phase, and hence, when the ammonium acetate was replaced with sodium acetate, an increase in transition metal ion retention at 60,

Table 4

Retention data for alkaline earth and transition metal ions on a 10 cm bare silica monolithic column with MeCN based mobile phases containing varying amounts of sodium acetate buffer—(pH 4.6)

log[E]	log k'					
	Ca(II)	Mg(II)	Sr(II)	Ba(II)		
70% MeCN						
–211	0.68	0.68	0.83	0.96		
–1.81	0.40	0.41	0.54	0.65		
–1.63	0.16	0.16	0.29	0.39		
–1.51	0.12	0.12	0.27	0.36		
Slope	–0.986	–0.988	–0.985	–1.046		
R ²	0.980	0.979	0.969	0.973		
	Cu(II)	Cd(II)	Ni(II)	Co(II)	Mn(II)	Pb(II)
60% MeCN						
–1.80	0.41	–0.09	0.06	0.13	0.16	0.61
70% MeCN						
–1.80	0.66	0.11	0.26	0.38	0.42	0.95
80% MeCN						
–1.99	–	0.56	0.80	1.07	1.20	–
–1.80	0.98	0.38	0.62	0.82	0.88	1.31
–1.69	0.82	0.27	0.52	0.71	0.76	1.11
–1.39	0.47	0.16	0.37	0.50	0.53	0.72
Slope	–1.211	–0.691	–0.701	–0.937	–1.080	–1.392
R ²	0.996	0.915	0.969	0.971	0.960	0.994

Flow rate = 1 mL/min and column temperature = 20 °C.

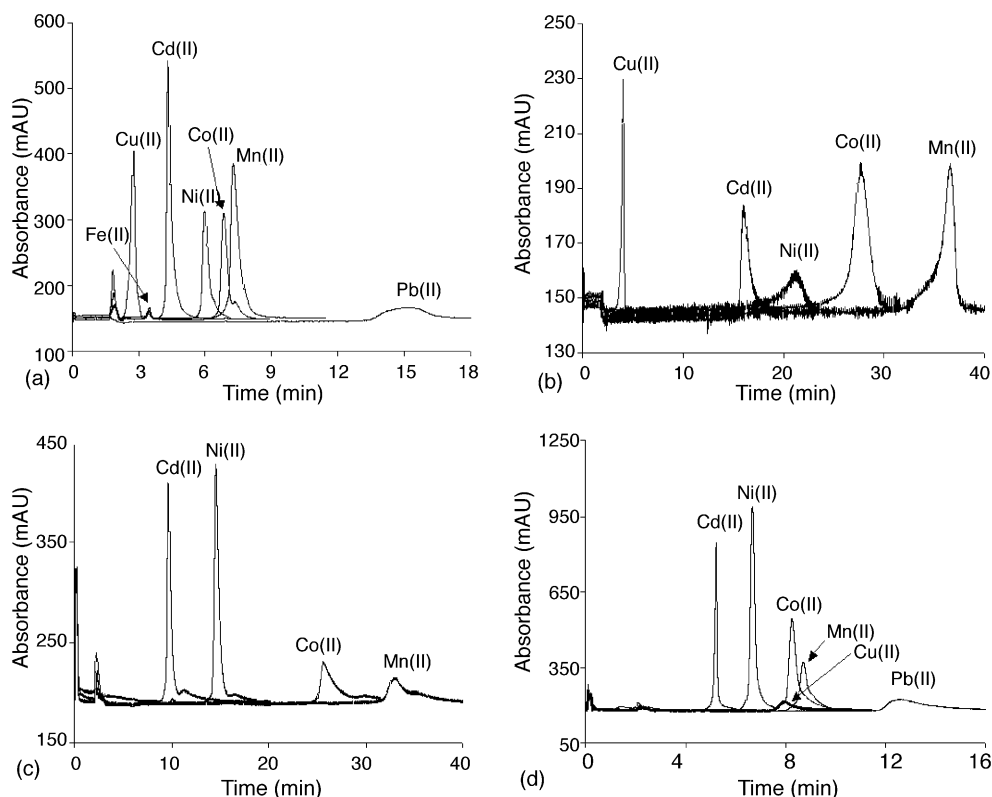


Fig. 2. Overlaid standard chromatograms of transition and heavy metal ions on a bare silica monolith using a mobile phase of: (a) 70% MeCN containing 15.6 mM ammonium acetate at pH 4.6; (b) 80% MeCN containing 5.2 mM ammonium acetate at pH 4.6; (c) 80% MeCN containing 10.3 mM sodium acetate buffer at pH 4.6; (d) 80% MeCN containing 41.2 mM sodium acetate buffer at pH 4.6, flow rate = 1 mL/min.

70 and 80% was observed. In particular, the behaviour of Cu(II) was interesting, considering the small retention of Cu(II) observed to be using the ammonium acetate buffer. In contrast, with the sodium acetate buffer, the retention of Cu(II) was significant and affected strongly by increasing the buffer concentration, i.e., at 15.5 mM sodium acetate buffer concentration, Cu(II) eluted almost 4 min after Mn(II), however, at 41.2 mM, Cu(II) was observed to elute before both Co(II) and Mn(II). Therefore, an increase in the sodium acetate buffer concentration from 10.3 to 41.2 mM resulted in a change in retention order from $\text{Cd(II)} < \text{Ni(II)} < \text{Co(II)} < \text{Mn(II)} \ll \text{Cu(II)} \ll \text{Pb(II)}$ to $\text{Cd(II)} < \text{Ni(II)} < \text{Cu(II)} < \text{Co(II)} < \text{Mn(II)} < \text{Pb(II)}$, as shown in Fig. 2(c and d). It is clear from this study and the previously results performed using MeOH in place of acetonitrile in the mobile phase, that the retention of Cu(II) is affected by both the formation of Cu(II)–MeCN complexes and complexation with ammonium in the mobile phase.

With the sodium acetate based mobile phase the optimum eluent conditions for the separation of the Cd(II), Ni(II), Co(II) and Mn(II) were found to be 80% acetonitrile containing 10.3 mM sodium acetate buffer (pH 4.6) (as in Fig. 2(c)). However, the run time required for the separation of these four analytes was greater than 35 min and as a result, excessive retention of Cu(II) and especially Pb(II) was observed, whilst using 80% acetonitrile/10.3 mM ammonium acetate

buffer, the separation of Cu(II), Cd(II), Ni(II), Co(II) and Mn(II) was possible in <20 min at 1 mL/min.

Using the sodium acetate buffer (as with the ammonium based mobile phase) there was still evidence of peak splitting for certain transition metal ions, especially at low buffer concentrations. For most cations this splitting was eliminated when using higher buffer concentrations, although was still present to a minor degree in some cases. Matching of the sample matrix with the mobile phase did affect the observed splitting effect to some degree but in an unpredictable manner, and is the subject of further investigation. In addition, the comparison of new and old silica monolithic columns was carried out to eliminate the possibility of column degradation being the reason for the observed splitting, and this comparison showed the effects to be reproducible between used and new columns.

3.7. pH effects

As with alkaline earth metals, it was found that the retention times of transition metal cations also varied considerably with mobile phase pH. Increasing mobile phase pH resulted in rapid increases in retention, such that when using an 80% MeCN mobile phase containing 10.3 mM of the ammonium acetate buffer at pH 5.6, the retention of Ni(II) was >1 h, and Co(II), Mn(II), Pb(II) and Zn(II) were all completely retained.

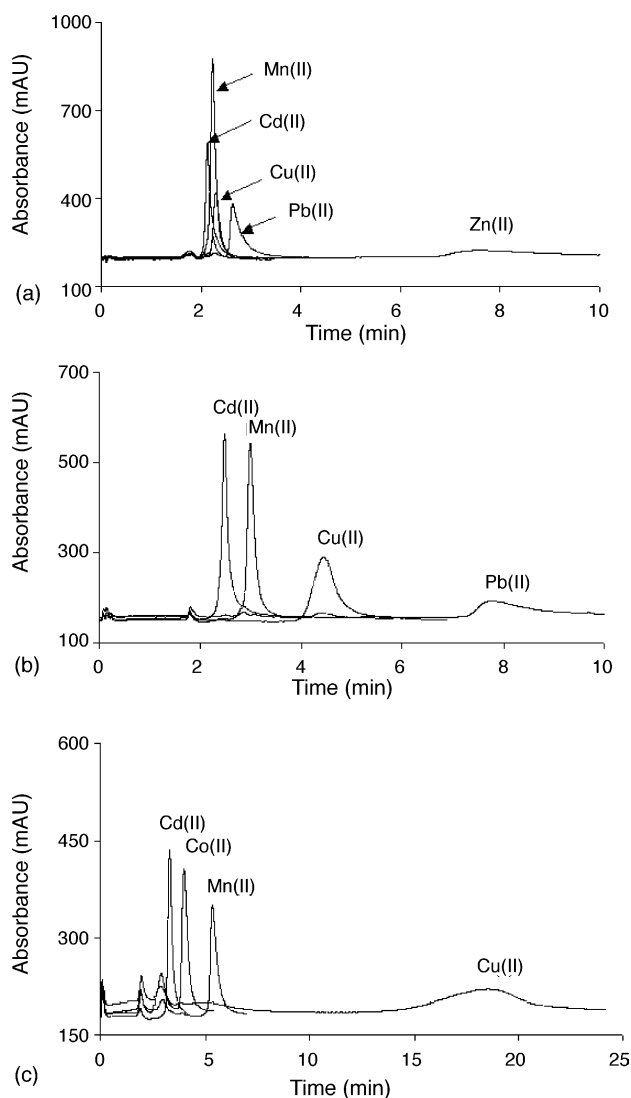


Fig. 3. Overlaid standard chromatograms of transition and heavy metal ions on a bare silica monolith using a mobile phase of: (a) 60% MeOH containing 21.1 mM ammonium acetate at pH 4.7; (b) 80% MeOH containing 10.3 mM ammonium acetate at pH 4.7; (c) 90% MeOH containing 5.1 mM ammonium acetate at pH 4.7, flow rate = 1 mL/min.

Alternatively, it was noted that reducing pH resulted in a considerable reductions in retention, resulting in the virtual co-elution of Cd(II), Ni(II), Co(II) and Mn(II) when using the 80% MeCN mobile phase at pH 3.5, with all metal ions eluting between 3.7 and 3.8 min. Therefore, a study of pH effects from pH 3.5 to 5.6 ($n = 5$) using the 80% MeCN mobile phase was carried out and in general it was found that both retention and resolution improved with increasing mobile phase pH. These retention data are shown graphically as Fig. 4. The graph shows most cations behave similarly, with the exception of Cu(II), which showed decreased retention at higher pH. Here, the explanation is thought to lie in the formation of amino complexes with ammonium ions within the buffer under high pH conditions.

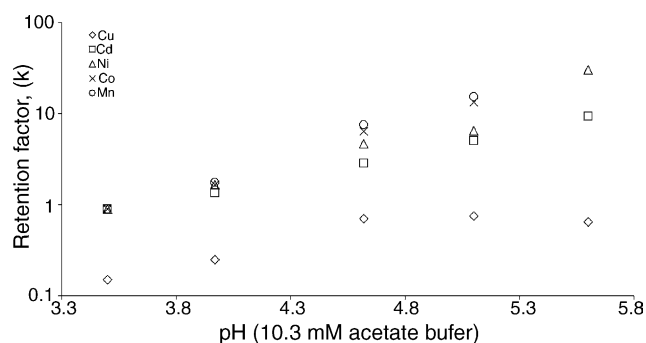


Fig. 4. Effect of mobile phase pH upon retention of transition metals on bare silica monolith using a mobile phase of 80% MeCN containing 10.3 mM ammonium acetate buffer, flow rate = 1 mL/min (at pH 5.6, Co(II) and Mn(II) were completely retained).

3.8. Flow rate effects

Obviously, a great deal of interest in monolithic columns for chromatographic purposes has been due to the potential for rapid separations. Here, using a mobile phase composition of 80% MeCN with 10.3 mM ammonium acetate (pH 4.6), the mobile phase flow rates were systematically increased from 1 to 5 mL/min. Optimal flow rate, in terms of measured peak efficiencies was found to be between 0.15 and 0.2 cm/s for most metals (corresponding to between ~1.5 and 2.0 mL/min). At a flow rate velocity of 0.2 cm/s, the average efficiency (HETP) for the five metals shown was found to be 40 μ m, ranging from 25.9 to 57.8 μ m, equivalent to an average efficiency of ~25,000 N/m (efficiency values calculated using peak widths measured at half height).

Fig. 5 shows cation separations run at flow rates of 1 mL/min, 3 mL/min and 5 mL/min, using the mobile phase conditions used for the flow rate study described above. Resolution of the five transition metal cations shown was clearly very acceptable, with possibilities to improve the separation further through the use of complexing eluents or gradient separations. With the use of the elevated mobile phase flow rate, which could be increased to >5 mL/min without excessive backpressure if required, transition metal separations can be

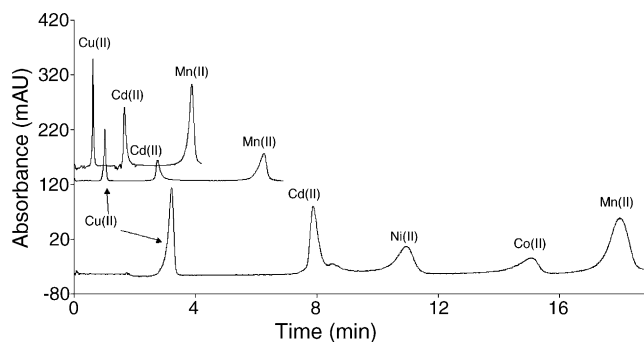


Fig. 5. Separations of selected transition metal ions on a bare silica monolith using a mobile phase of 80% MeCN containing 10.3 mM ammonium acetate buffer at pH 4.6 at flow rates of 1 mL/min, 3 mL/min and 5 mL/min.

achieved in as little as 2–3 min, with acceptable resolution and peak shapes, as illustrated by Fig. 5.

4. Conclusions

This study shows that bare silica monoliths can be used for efficient and practical separations of alkaline earth and transition metal cations, without surface modification, through the use of organic based mobile phases, in what can be best described as solvent enhanced ion chromatography. The organic nature of the mobile phase enhances the electrostatic interaction of the analyte ions and the fixed ion-exchange site, in this case the dissociated silanol groups, through reducing the associated number of waters of hydration. However, this effect is dependent upon the nature of the organic solvent and significant selectivity changes can be obtained through correct choice of solvent. The assumption that the observed retention is simply enhanced ion exchange is verified by the effects of both pH and buffer type and concentration, which are as expected for a simple ion-exchange system. Finally, the unique selectivity of Chromolith bare silica monolithic columns for Zn(II) and is worthy of further investigation.

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References

- [1] H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, *Anal. Chem.* 68 (1996) 3498.
- [2] F. Svec, *J. Sep. Sci.* 27 (2004) 747.
- [3] D. Connolly, D. Victory, B. Paull, *J. Sep. Sci.* 27 (2004) 912.
- [4] D. Victory, P. Nesterenko, B. Paull, *Analyst* 129 (2004) 700.
- [5] P. Hatsis, C.A. Lucy, *Analyst* 127 (2002) 451.
- [6] P.N. Nesterenko, M.A. Rybalko, *Mendeleev Commun.* 14 (2004) 121.
- [7] B. Paull, C. O'Riordan, P.N. Nesterenko, *Chem. Commun.* (2005) 215.
- [8] J. Hutchinson, P. Zakaria, A.R. Bowie, M. Macka, N. Avdalovic, P.R. Haddad, *Anal. Chem.* 77 (2005) 407.
- [9] P. Zakaria, J.P. Hutchinson, N. Avdalovic, Y. Liu, P.R. Haddad, *Anal. Chem.* 77 (2005) 417.
- [10] Q. Xu, K. Tanaka, M. Mori, M.I. Helaleh, H. Toada, W. Hu, K. Hasebe, *Chromatographia* 57 (2003) 19.
- [11] E. Sugrue, P. Nesterenko, B. Paull, *J. Sep. Sci.* 27 (2004) 921.
- [12] E. Sugrue, P. Nesterenko, B. Paull, *Analyst* 128 (2003) 417.
- [13] P.N. Nesterenko, B. Paull, *Trends Anal. Chem.* 24 (2005) 295.
- [14] B.W. Pack, D.S. Risley, *J. Chromatogr. A* 1073 (2005) 269.
- [15] K. Ohta, M. Sando, K. Tanaka, P. Haddad, *J. Chromatogr. A* 752 (1996) 167.
- [16] K. Ohta, K. Tanaka, B. Paull, P. Haddad, *J. Chromatogr. A* 770 (1997) 219.
- [17] K. Ohta, K. Tanaka, *Anal. Chim. Acta* 381 (1999) 265.
- [18] E. Munaf, R. Zein, T. Takeuchi, T. Miwa, *Anal. Chim. Acta* 334 (1996) 39.
- [19] E. Munaf, R. Zein, T. Takeuchi, T. Miwa, *Anal. Chim. Acta* 341 (1997) 225.
- [20] R.L. Smith, D.J. Pietrzyk, *Anal. Chem.* 56 (1984) 610.
- [21] Y. Takeda, K. Ishida, T. Hasegawa, A. Katoh, *J. Chromatogr. A* 1049 (2004) 233.
- [22] G.J. Moody, J.D.R. Thomas, *Analyst* 93 (1968) 557.
- [23] B. Paull, P.N. Nesterenko, *Analyst* 130 (2005) 134.
- [24] A.I. Elefterov, M.G. Kolpachnikova, P.N. Nesterenko, O.A. Shpigun, *J. Chromatogr. A* 769 (1997) 179.
- [25] P. Janos, K. Stulik, V. Pacakova, *Talanta* 38 (1991) 1445.
- [26] D.L. Dugger, J.H. Stanton, B.L. McConnel, B.N. Irby, W.W. Cummings, R.W. Maatman, *J. Phys. Chem.* 68 (1964) 757.
- [27] E. Akatsu, R. Ono, K. Tsukuechi, H. Uchiyama, *J. Nucl. Sci. Technol.* 2 (1965) 141.
- [28] B.N. Laskorin, V.V. Strelko, D.N. Strahesko, V.I. Denisov, *Silica Gel-Based Sorbents in Radiochemistry*, Atomizdat, Moscow, 1977.
- [29] G.C. Bye, M. McEvoy, M.A. Malati, *J. Chem. Soc. Faraday Trans. 1* (79) (1983) 2311.
- [30] H.H. Tran, F.A. Roddick, J.A. 'Donnel, *Water Res.* 33 (1999) 2992.
- [31] P.W. Schindler, B. Fürst, R. Dick, P.U. Wolf, *J. Colloid Interface Sci.* 55 (1976) 469.
- [32] R.K. Iler, *The Chemistry of Silica. Solubility, Polymerization, Colloid and Surface Properties and Biochemistry*, A Wiley/Interscience Publication, New York/Chichester/Brisbane/Toronto/Singapore, 1979.
- [33] E. Sugrue, P.N. Nesterenko, B. Paull, *J. Chromatogr. A* 1075 (2005) 167.
- [34] V.N. Vandyshev, *Russ. J. Gen. Chem.* 73 (2003) 855.